OBSERVATIONS ON THE COLOR CHANGES AND ISOLATED SCALE ERYTHROPHORES OF THE SQUIRREL FISH, HOLOCENTRUS ASCENSIONIS (OSBECK)

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On transference from a dark to a light-colored background the common Bermudian squirrel fish, *Holocentrus ascensionis* (Osbeck), shows an exceptionally rapid color change from red to white. This change is coincident with and the result of a pigment concentration within the numerous erythrophores of the scales and the deeper layers of the skin. The speed with which this is effected is strikingly demonstrated when a red fish from a dark background (in these experiments such backgrounds were usually black) is placed in a white aquarium containing three or four animals in the pale condition. In five seconds the originally red fish is indistinguishable from the earlier occupants. Similarly a pale fish placed in the company of red fishes in an aquarium over a black background loses its distinctive coloring in about ten seconds. Such rapid color changes are rarely met with among the fishes, at least under laboratory conditions, although they are not uncommon among the lizards. In the scorpion fish of the Bay of Naples, *Scorpæna ustulata*, a red form comparable in color to *Holocentrus* with erythrophores as the dominating type of pigment cell, the shift from red to the pale condition is a matter of hours (Smith and Smith, 1934). In melanophores the change in distribution of the pigment granules in response to a variation in background from white to black or vice versa is usually completed in three to four minutes. The melanophores of *Fundulus heteroclitus* behave typically in this manner.

The rapidity with which *Holocentrus* is capable of altering the distribution of pigment within its erythrophores is suggestive of a high degree of nervous control over the activities of the chromatophores. Such a supposition is borne out by experiments based upon the methods originally employed by Pouchet (1876), von Frisch (1911, 1912) and others in working out the relationship between the nervous system and chromatophores of fishes. These methods rely principally upon sectioning the sympathetic chain at various levels along its course and noting the effect of such operations upon the subsequent behavior of the pig-

ment cells in the area affected by the operation. In *Holocentrus* if the section involves the anterior half of the sympathetic chain, the area in front of the cut turns red and stays red regardless of background. If the cut, on the other hand, involves the posterior half of the chain the reddened area is confined to the regions behind the cut. In *Holocentrus* severance of the sympathetic then results in a typically persistent expansion of the denervated erythrophores, the distribution of the pigment-motor fibers to these erythrophores conforming to the scheme originally announced by von Frisch (1911) as applying to the innervation of the melanophores of *Phoxinus lævis*. In both species the pigment-motor fibers emerge from the spinal cord at a point about halfway down the trunk and are distributed both anteriorly and posteriorly by way of the sympathetics to the chromatophores in question. Operations of the nature just described demonstrate clearly the existence of sympathetic pigment-motor fibers capable of governing the responses of the erythrophores of *Holocentrus*.

In operated fishes kept upon a white background the resultant red or denervated areas produced by cutting the posterior sympathetic chain were visible in whole or in part for ten or fifteen days after the operation. In such denervated regions the first sign of recovery of function on the part of the affected erythrophores appeared in those cells lying most anteriorly in the operated area. In animals kept upon a white background this portion paled within three or four days after the operation, the paling gradually spreading each day more and more posteriorly until finally the whole area had lost its red color. Such animals when placed on a dark background showed a uniform reddening over the entire body. Repetition of the operation at its original site led to an exact duplication of the original reddening. This reëstablishment of the denervated condition following a second operation together with the nature of the recovery from the original operation is not in disagreement with the assumption that there is a gradual regrowth of nerves into the denervated region together with reëstablishment of nervous connections with the erythrophores. Denervations of the erythrophores of the anterior half of the trunk present a different picture. Here the recovery is much more rapid, so rapid in fact that the possibility of nervous regeneration is excluded. Subsequent operations at the original site often failed to produce the original results. In fact, the general impression received after comparing the behavior of denervated areas in the head and anterior trunk with those in the posterior trunk strongly suggest some essential difference between the innervation of the erythrophores, or else, what seems even less likely, a structural or functional difference between the erythrophores in these two parts of the body. Unfortu-

nately the opportunity was not given us to pursue this matter further. A somewhat similar relationship between dorsal and ventral trunk melanophores in *Phoxinus* has also been reported (Smith, 1931).

Further proof of the nervous control of the erythrophores in *Holocentrus* is obtainable on stimulating the anterior end of the medulla where, following the work of von Frisch (1911), one would expect to find the pigment-motor center. When by means of a suitable stimulating electrode this is done in *Holocentrus* the entire animal immediately pales and stays pale as long as the stimulus is applied. On stopping the stimulation the animal reddens, the erythrophore pigment assuming the state which typically follows the necessary operative procedure involved in exposure of the posterior portion of the brain. The original paling may be reproduced at will as long as the chromatophores remain in a reactive condition. Furthermore stimulation of the opthalmic nerve at

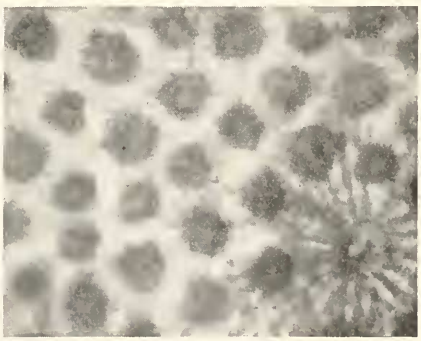

FIG. 1. Pulsating erythrophores in the expanded phase in N/10 NaCl. A non-pulsating expanded melanophore in the lower right-hand corner.

a point where it crosses the superior surface of the orbit produces a marked ipsilateral paling of the head (Pouchet, 1876; v. Frisch, 1911; Smith, 1931).

A reversal of the effect of temperature changes on the erythrophores of the trunk before and after sectioning the sympathetic chain gave further evidence of an innervation of these cells. Exposure of the unoperated trunk to a locally directed stream of warm sea water (35° C.) produced in the affected region a pronounced expansion of the erythrophores, as shown by the increase in the red color of the surface of the body at the point warmed. This reddening was strictly confined to the area heated. If, however, the stream were directed upon an area of the trunk in the same fish denervated a few days previously the erythrophores now contracted and the heated area became pale. A similarly directed stream of cold water produced in the innervated trunk a con-

traction and in the denervated trunk an expansion of the erythrophores. This curious reversal of the responses to heat and cold on the part of certain chromatophores after denervation has previously been noted in connection with the melanophores of *Fundulus heteroclitus* (Smith, 1928). Such reversals are not apparently the rule among fishes as attempts on other occasions to evoke the same response in the chromatophores of other forms (*Phoxinus lævis, Scorpæna ustulata, Trigla* sp.) have been unsuccessful, the direction of the response of the chromatophores of these fishes to temperature changes remaining the same whether denervated or innervated. This was also true with another Bermudian form, the surgeon fish, *Acanthurus hepatus*. The extent to which this peculiarity characteristic of the chromatophores of *Fundulus* and *Holocentrus* is spread among other fishes should be a matter worth investigating further.

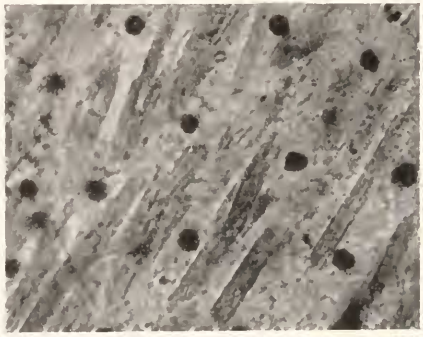

FIG. 2. Pulsating erythrophores in the contracted phase in N/10 NaCl.

The scales of the squirrel fish upon microscopical examination proved to be favorable objects for direct observations upon the activity of the chromatophores. Three types were present, erythrophores, xanthophores, and melanophores, the erythrophores being the most numerous with an even distribution throughout the pigmented area of the scale, while the xanthophores, less abundant than the erythrophores, were largely confined to the distal corners. The melanophores were relatively scarce and were more or less irregularly scattered throughout the scale. In sea water isolated scales showed chromatophores in the semi-expanded condition, that is with only the proximal one-third or less of the processes of the cell filled with pigment. In N/10 NaCl, however, the pigment distribution was no longer a static affair, the erythrophores and the xanthophores instead alternating between states of expansion and contraction, the pigment granules migrating rhythmically in and

out of the processes (Figs. 1 and 2). These pulsations first appeared at the proximal border of the pigmented area, occurring almost simultaneously with the color changes of the iridocytes (Smith, 1933). This would indicate that the pulsations were an immediate response to the penetration of the NaCl into the tissues of the scale. The pulsations were relatively slow at first, beginning with two or three a minute, a level from which they gradually increased to between fifteen or twenty-five a minute. At this point the rate either gradually declined until pulsations ceased entirely or else it continued to increase further. In this latter event rates of fifty to sixty a minute were not uncommon, the extent of the pulsations becoming progressively less and less until it was almost impossible to tell whether the cells were pulsating at all, the erythrophores apparently remaining contracted. Usually after the erythrophores had ceased pulsating a period of quiescence set in lasting from three to five minutes, followed by a second and on occasion even a third or fourth period of activity. Thus the time in which the pulsatory activity could be seen usually lasted forty-five minutes to an hour and a half, although now and then individual cells were seen to pulsate for three hours or more, long after all the rest of the erythrophores in the scale had become quiet. During the first few minutes of the pulsations the movements of all the erythrophores as well as the xanthophores were synchronous throughout the scale. As time went on, however, the chromatophores comprising the pigmented area broke up into smaller groups, each group proceeding to pulsate at a rhythm independent of the others. This synchronism might indicate some sort of coördinating mechanism within the scale. The rate and extent of the erythrophore pulsations in one scale, however, compared with another were extremely variable, scarcely any two scales being even approximately alike.

The failure of the melanophores to pulsate under conditions which excite the erythrophores and xanthophores to activity does not indicate that these cells are incapable of such activity. Pulsations may be induced in the melanophores by use of a method originally introduced by Spaeth (1916). This requires a five-minute exposure of the cells to N/10 $BaCl_2$ followed by a transfer of the scale to N/10 NaCl. In *Holocentrus* scales so treated the melanophores show good pulsations in about five minutes after the immersion in NaCl following treatment with $BaCl_2$. These pulsations differ from those seen in the erythrophores and the xanthophores in that they are much slower, occurring only once or twice a minute. Slow pulsations of this sort are also characteristic of the melanophores of *Fundulus heteroclitus* in which such activity has been extensively studied (Spaeth, 1916; Smith, 1930).

Erythrophore pulsations have been reported before. In fact this

type of behavior on the part of chromatophores was first observed in connection with the erythrophores of *Mullus*. Ballowitz (1913) described rhythmical movements of the pigment granules in the erythrophores of excised bits of the skin of this form when such pieces of the skin are immersed in 0.75 per cent NaCl. Judging from his descriptions, erythrophore pulsations in this form were of the slow type seen in the melanophores of *Fundulus*. The isolated scale erythrophores of *Scorpæna ustulata* can also be made to pulsate (Smith and Smith, 1934) but only after they have been previously treated with $BaCl_2$. In this respect the *Scorpæna* erythrophores resemble more the melanophores of *Fundulus* and *Holocentrus* than the erythrophores of *Holocentrus*. In *Scorpæna* the erythrophores pulsate steadily for an hour or so at a rate of about once every two minutes. Melanophores in the same scale pulsate somewhat more rapidly, about once a minute on the average.

FIG. 3. Showing the progressive contraction of the chromatophores in N/10 KCl after sea water. Cells in the lower right contracted, cells in the upper left still expanded. The small bodies are erythrophores and the larger ones melanophores.

When *Holocentrus* scales are placed in N/10 KCl all of the chromatophores show a marked contraction (Fig. 3). This is quite in keeping with the results of other investigators upon the effect of KCl upon isolated scale chromatophores. In $CaCl_2$ the erythrophores and xanthophores of *Holocentrus* show a brief period of pulsatory activity lasting about eight minutes, after which they assume the contracted condition. That the pulsations were occurring at a time when the cells were under the influence of the $CaCl_2$ is shown by the fact that the iridocytes throughout the entire scale were colorless. Attempts were also made to study the effects of various drugs upon erythrophore pulsations but the results were not worthy of serious consideration since no adequate means of controlling the experiments could be found. Comparison between two scales, even adjoining scales from the same fish,

were quite useless as there was no constant uniformity in respect to the erythrophore behavior of the two. Scales were even cut into two halves, one half being immersed in solutions of the drug to be tested and the other half in NaCl as a control, but again no reliance could be placed on the data from a quantitative standpoint as control experiments showed that the erythrophores of the two halves of the same scale when placed in NaCl differed markedly in their behavior.

From these results and the results of previous investigation (von Frisch, 1912; Smith and Smith, 1934) it is apparent that in certain fishes the erythrophores are clearly under nervous control. In forms such as *Phoxinus* (Giersberg, 1930) it is equally clear that the erythrophores are independent of nervous control and are governed in their reactions by humoral factors. It still remains to be seen whether this bears any relation to the fact previously noted by us that in those forms where the erythrophores are under partial or complete humoral control their principal function seems to be their contribution to the assumption of the nuptial coloration shown during the spawning season. At other times they are relatively inactive. In forms such as *Holocentrus* and *Scorpæna* where a nervous control has been demonstrated the erythrophores are continually active at all seasons of the year and are in fact the dominating pigment cell among the chromatophores these animals possess.

In *Holocentrus* the erythrophores and xanthophores are alike in their behavior, both types of cells reacting about the same throughout all of the experiments attempted although possibly the xanthophores were somewhat more sluggish than the erythrophores in their responses to temperature changes. In *Scorpæna,* however, the xanthophores differ markedly from the erythrophores in that they show no pulsations in NaCl after treatment with $BaCl_2$ as do the erythrophores and are also much more sluggish in regard to their responses to other types of stimuli. Schnakenbeck (1925), after an examination of eighty different species of fish, came to the conclusion that there was no fundamental difference between xanthophores and erythrophores, in many cases both types of pigment being present in the same cell. On physiological grounds, however, such a generalization does not appear to be justified, although it may well be true of some fishes (i.e., *Holocentrus*). Any comparison between erythrophores and xanthophores other than in the same species seems useless until it is definitely established whether or not there are two distinct types of erythrophores, first the sluggish humorally controlled kind found in *Phoxinus* and second the highly sensitive nervously controlled type found in *Holocentrus.* Whether the difference between the two is actually clean cut, or whether the apparent

distinction is only illusory as a result of the study of two extreme types in a graded series is a matter for further investigation.

Further complexity is again suggested when the melanophores and the erythrophores are compared. In *Scorpæna* the two types of pigment cells seem to behave essentially the same, differing only on morphological grounds. In *Holocentrus,* on the other hand, the melanophores vary pronouncedly from the erythrophores not only in appearance, for this they do also in *Scorpæna,* but also in responsiveness to certain types of stimuli. In *Holocentrus* the melanophores do not pulsate spontaneously in NaCl when transferred directly from sea water. In this species the erythrophores were by far the most active type of pigment cell present in the scale. The melanophores appeared to be sluggish in comparison, although the melanophores of *Holocentrus* were no more sluggish in their behavior than the melanophores of other forms. But the extreme sensitivity of the erythrophores of *Holocentrus* might well be anticipated after witnessing the rapidity with which this animal can adapt itself to a new shade of background. Apparently this form possesses a pigment cell which is fully as responsive as the chromatophores of lizards and in fact approaches the sensitivity of the entirely distinct pigmentary effector organ of the cephalopod.

Summary

1. In the common Bermudian squirrel fish, *Holocentrus ascensionis* (Osbeck), sympathetic pigment-motor fibers can be demonstrated which are capable of altering the state of pigment distribution within the erythrophores.

2. In the denervated trunk erythrophores of *Holocentrus* an increase in temperature will cause a withdrawal of the pigment into the central body of the cell. Lowering the temperature has the opposite effect. In innervated trunk erythrophores the effect of temperature changes upon the state of pigment distribution of the erythrophore is the reverse of what is seen in denervated erythrophores.

3. Isolated scale erythrophores and xanthophores of *Holocentrus* show spontaneous pulsations when transferred from sea water to N/10 NaCl. Isolated scale melanophores will not pulsate in NaCl unless previously treated with N/10 $BaCl_2$.

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